future studies is how different signals from the microenvironment that are sensed through distinct G protein-coupled receptors, such as Ffar2 and GPR183, are integrated by ILC3s in the colon. Regardless, the findings by Chun et al. potentially open up new therapeutic possibilities. Defects in the epithelial barrier are thought to contribute to inflammatory bowel disease (IBD) in humans, and Ffar2 agonism, e.g., through SCFA administration, could restore intestinal barrier function (Parada Venegas et al., 2019). Furthermore, it will be worth examining whether polymorphisms in *FFAR2* confer risk to develop IBD. Only the future will tell how Ffar2 ILC3s will go with the help of a little fiber digested by gut bugs.

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Building a Human Thymus: A Pointillist View

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https://doi.org/10.1016/j.immuni.2019.10.003

In this issue of *Immunity*, Zeng et al. use single-cell RNA sequencing analyses of rare samples to shed light on the emergence of thymic stromal cell types, the first developing T lymphocytes, and their possible pre-thymic precursors in the early human fetus.

In this issue of *Immunity*, Zeng et al. present the first glimpses of how individual cells in the early human fetus develop into a thymus and begin to produce T cells (Zeng et al., 2019). The thymus is the "factory" in which small numbers of immigrating blood-cell precursors are driven through extensive proliferation and differentiation to generate T cells of diverse types. Both the flexible, varied differentiation programs of the T cells themselves and the ontogeny of the epithelial and mesenchymal compartments that eventually guide the developing lymphocyte precursors to their fates need illumination. Zeng et al. use single-cell RNA sequencing (scRNA-seq) data acquired from multiple tissue sources and time points to distinguish the earliest stages of fetal human T cell development. Their data show how $\alpha\beta$ T cell receptor (TCR)-expressing T cells diverge in their development from $\gamma\delta$ TCR-expressing ones and suggest affiliations with cells in the fetal liver that may be related to thymocyte precursors. The early human fetal thymus is also shown to contain populations of innate lymphoid cell type (ILC)1, ILC3, and innate-like T-lineage cells as well as conventional T cells. Finally, the authors characterize the differentiation of distinct sets of thymic stromal cells and provide inference of signaling ligand-receptor pairs between cell types that may organize the stroma itself as well as mediate communication between stroma and lymphocytes.

T cell developmental stages have been intensely studied and are generally well distinguished by combinations of cell-surface markers, which broadly correlate with stereotyped gene expression changes (Yui and Rothenberg, 2014). In the mouse, the stage markers have been validated by *in vivo* and *in vitro* transfer experiments, clonal differentiation assays under distinct environmental conditions, and targeted genetic perturbation studies. However, individual T cell precursors in the same thymic cohort can have varied

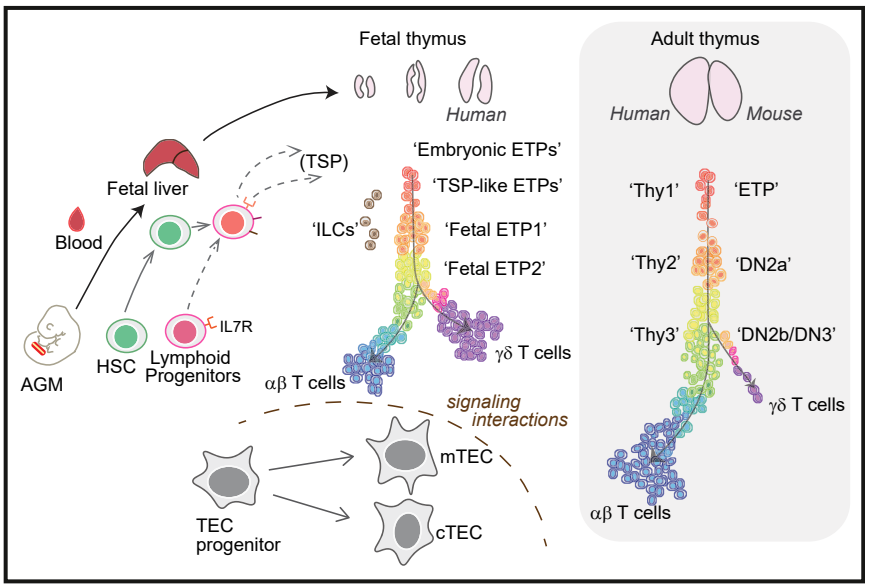

Figure 1. Single-Cell Analysis of Human Thymus Populations and Candidate Pre-thymic Precursors in Early Fetal Stages
(Left) Potential sources of thymus-seeding immigrants analyzed.
(Middle) Cell types in human fetal thymus from 8 to 10 weeks of gestation.
(Right) Approximate correspondence with previously characterized T cell precursors in postnatal mouse and human thymus. Thymocyte colors indicate similar transcriptomes.
(Bottom) Characterization of thymic epithelial differentiation and potential intercellular signaling pathways. Dashed arrows, lineage uncertain. Distinctions between thymic subsets that are inferred primarily from cell-cycle signatures contribute strongly to calculated trajectories in Zeng et al. but are not included here. AGM, aortic-gonadal-mesonephric region, site of first definitive hematopoietic stem cells; HSC, hematopoietic stem cell; ETP, early T cell progenitor; DN, double negative $CD4^{-}$ $CD8^{-}$ thymocyte.

developmental potentials and can go on to divergent fates. What molecular mechanisms control these different developmental outcomes? As single-cell methods for genome-wide RNA expression analysis (transcriptomics) have become accessible, multiple groups have started applying this approach to dissect the early stages of thymic development and the components of the thymic stroma. The work of Zeng et al. is in the vanguard of such studies on human thymic ontogeny, following a comprehensive, dynamic single-cell analysis of hematopoietic and stromal cells during thymic organogenesis in the mouse fetus (Kernfeld et al., 2018) and complemented by single-cell dissections of thymic stromal cell types (Bornstein et al., 2018) and very early T-lineage substages (Zhou et al., 2019) in postnatal mice.

Zeng et al. have analyzed three samples of human thymus from 8, 9, and 10 weeks of gestation and other candidate pre-thymic hematopoietic tissues. These rare samples are a tour de force to obtain, and the authors have sought to extract the maximum information from single-cell transcriptome measurements and application of bioinformatic clustering and trajectory inference methods, alone. Necessarily, this leaves much work ahead for confirmation and clarification, and some provocative points made by the authors need to be considered in this light. However, the data provide good evidence for the rapid establishment of T cell development in these human samples, the early presence of ILC-like cells, and a resemblance between the transcriptomes of lymphoid precursors from fetal liver and those of the first intra-thymic T cell precursors. While the cells in the 8-week thymus appear primitive and substantially unlike later thymocytes, the 9-week gestation thymus already contains divergent αβ and γδ T lineage cells, as well as molecularly distinct thymic cortical and medullary epithelial cells. In comparison, the mouse thymus only begins to be populated around day 12 out of 20, as late relative to total gestation as a human fetus at 24 weeks.

The early T cell differentiation stages defined by Zeng et al. appear to progress from initial immigrants with multiple non-T-like features through upregulation of T-lineage genes and then divergence to αβ and γδ lineages, in general agreement with bulk population data (Ha et al., 2017). One caveat is needed: the nomenclature Zeng et al. use is potentially confusing, as the term "ETP" is applied unusually broadly. "ETP" has been well-established to refer to the earliest intra-thymic stage(s), before expression of any definitive T cell markers (Allman et al., 2003; Ramond et al., 2014; Zhou et al., 2019). However, based on their transcriptomes, the subsets called "ETP" by Zeng et al. appear to span all stages of T cell development up to TCR gene expression, i.e., corresponding to ETP, DN2a, and DN2b stages in postnatal mice (Yui and Rothenberg, 2014); Tconv1 and 2 in fetal mice (Kernfeld et al., 2018); or Thy1, Thy2, and Thy3 in postnatal human T cell development (Ha et al., 2017)(Figure 1). Given the rarity of the samples used here, it has not been possible to test all the functional developmental relationships of these subpopulations by precursor-product analyses or single-cell assays of developmental potential and progression, as one can for the mouse (Ramond et al., 2014; Wada et al., 2008; Zhou et al., 2019). Thus, correspondences with previously characterized subsets (Figure 1) remain to be confirmed.

Interestingly, on transcriptome evidence, the authors do find hints that differentiation may proceed faster for individual cells in the fetal thymus than it may in thymocytes after birth (Figure 1), since prominent cell clusters apparently co-express both early and later gene signatures. Genes associated with later (post-commitment) stages of mouse T cell development, including *Bcl11b*, the *Cd3* genes, *Cd4*, *Cd8a*, and *Cd8b*, already seem to be expressed by early-fetal human precursors in clusters that are still predicted to have an otherwise primitive cell-surface marker phenotype (CD34+ CD7+ CD1a−). Cluster definition in single-cell analysis is always provisional, and gene expression levels averaged across a clustered population, as in the results shown (Zeng et al., 2019), might not apply to every cell in the cluster. Still, the data suggest that in this initial human T cell developmental wave there

may be an accelerated activation of later T-lineage genes before the cells lose their early-precursor phenotype. Note that in the mouse, too, regulatory changes during first-wave fetal T cell development appear to occur faster than in postnatal waves of T cell development (Belyaev et al., 2012). Further analysis of individual cell phenotypes in the data presented here could be very instructive about the order in which specific changes occur in this phase of T cell development.

While the 8-week thymus is shown to contain a high proportion of ILCs that are subsequently diluted out, in the 9- and 10-week gestation thymus there is profuse generation of γδ cells with distinctive transcriptome features (Zeng et al., 2019). The gene expression divergence between γδ and αβ lineage cells is particularly well demonstrated. The prevalence of γδ T cell production in the first wave of fetal T cell development, shown here (Figure 1), links human with other mammalian and non-mammalian species.

Finally, the transcriptomes of the thymic stromal components enable their development to be tracked and even potential new functions deduced. Although medullary thymic epithelial cell (mTEC) precursors do not yet activate expression of the transcription factor AIRE detectably by week 10, the cortical thymic epithelical cell (cTEC) precursors expand and activate strong expression of genes known to be important for their functions, consistent with results from the fine-grained time course study in mice (Kernfeld et al., 2018). Interestingly, putative mTEC and cTEC precursors also show notable differences in expression of signaling pathway genes: expression of BMP signaling and Hippo signaling components is biased toward mTEC precursors, while expression of the Notch ligand DLL4 and of Wnt signaling, tricarboxylic acid (TCA) cycle, and respiratory electron transport genes is biased toward cTEC precursors at these stages. By systematically examining expression of possible ligand-receptor signaling pairs in thymic lymphocytes and stromal components, the authors identify possible stromal-stromal interactions for morphogenesis as well as potential new sources, besides TECs, of microenvironmental influence on the developing T cells. They suggest that endothelial cells may supply adhesive interactions with developing lymphocytes via CD34 and L-selectin, while mesenchyme could be important as the source of insulin-like growth factor-1 (IGF1) and IGF2.

The picture of thymus ontogeny in the early human fetus emerging from the work of Zeng et al. thus provides rich clues, not only to possibly unique features of the lymphoid precursors that develop there but also to the stromal interactions that first establish the thymic architecture. Their valuable data will certainly reveal more about these stages as they are analyzed further and related more explicitly to other sources of relevant evidence. We are on the threshold of a major step forward in our understanding of human T cell development.

ACKNOWLEDGMENTS

Relevant work in the Rothenberg group has been supported by grants from the US Public Health Service: R01 HD076915, R01 HL119102, and R01 HD100039.

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